SCREENING OF RICE GENOTYPES FOR WATER AND SALINITY STRESS TOLERANCE

ABSTRACT

Experiments were undertaken to study the effect of drought and salinity on growth of various genotypes of rice. Listed, IET 26871, IET 26478, IET 26910, 26866, IET 26908, IET 26493, IET 26917, IET 26913; BPT 2766, BPT 2593, BPT 2595, BPT 2782, BPT 2776, BPT 2411, KARJA747, MEL26487, LALAT, GONTRABADAN, KRH4, US312, SAHABAGYADHAM, PA6129, PA6444, PR 113, MTU1010 and HRI174 lines were used and CB06-803, CB13-804, CB13-805 and TNRH 55from TNAU were taken for this study. Germination and seedling characters were measured at seventh day after inducing abiotic stress conditions such as, drought by application of mannitol at 1% and 2% and salinity by application of NaCl at 200mM concentration. Physiological and biochemical parameters were estimated at the twentieth day after inducing the drought and salinity stress conditions. The number of seeds germinated, shoot and root length, vigor index, nitrate reductase, α-amylase content and the soluble protein content were studied under the laboratory conditions in comparison to the control. Analyses of variance, revealed significant differences for germination percentage, shoot and root length, vigor index, nitrate reductase, α-amylase and protein contents, for mannitol levels as well as for sodium chloride. It was evident from the data that increased levels of mannitol and sodium chloride decreased the germination percent, shoot and root lengths and hence the vigor index nitrate reductase, α-amylase and protein contents linearly. The lesser reduction was noticed in the genotypes US 312, KRH-4, CB06-803, CB13-804 and PA-6444 (14 to 23%) due to increased levels of mannitol and sodium chloride over control. The highest reduction was recorded in Sahabhagidhanand HRI174 (28 to 37%) among the rice genotypes studied.

Key Words: Physiology-Water – Salinity – Stress tolerance – Rice genotypes.

INTRODUCTION

Rice is the seed of the grass species *Oryza sativa* (Asian rice) or *Oryzaglaberrima*(African rice). As a cereal grain, rice, a monocot, is normally grown as an annual plant, although in tropical areas it can survive as a perennial and can produce a ratoon crop for up to 30 years. The rice plant can grow to 1 to 1.8 m tall, occasionally more depending on the variety and soil fertility. It has long, slender leaves 50 to 100 cm long and 2 to 2.5 cm broad. The small wind-pollinated

UNDER PEER REVIEW

flowers are produced in a branched arching to pendulous inflorescence 30 to 50 cm long. The edible seed is a grain, a fruit called caryopsis 5 to 12 mm long and 2 to 3 mm thick.

Rice cultivation is well-suited to countries and regions with low labor costs and high rainfall, as it is labor intensive to cultivate and requires ample water. However, rice can be grown practically anywhere, even on a steep hill or mountain area with the use of water-controlling terrace systems. The traditional method for cultivating rice is flooding the fields while or after setting the young seedlings. This simple method requires sound planning and servicing of the water damming and channeling, but reduces the growth of less robust weed and pest plants that have no submerged growth state, and deters vermin. While flooding is not mandatory for the cultivation of rice, all other methods of irrigation require higher effort in weed and pest control during growth periods and a different approach for fertilizing the soil.

Water management Uniform leveling of field and proper drainage are most essential for an effective water management in irrigated field. Efficient water- management facilitates good tillering and better nutrient uptake and helps in reducing weed population.

In India rice is grown in 43.86 million ha, the production level is 104.80 million tones and the productivity is about 2390 kg ha^{-1} (Agricultural Statistics at a glance, 2015). The rice production has registered an appreciable increase from 20.58 million tonnes in 1950-51 to 104.86 million tonnes during 2014-15, which is nearly 5 times. The yield was 668 kg ha^{-1}in 1950-51 which has increased to 2390 kg ha^{-1}during 2014-15. Major share of rice production is in Kharif season. It is grown in almost all the states in the country however the major 5 states in rice production are West Bengal, UP, Andhra Pradesh, Punjab and Tamil Nadu.

It is one of the most important food crops and feeds more than 60 per cent population of India. Though the area in production of rice is high compared to that of china, the production and productivity of rice is low in India. And also the productivity of countries like Indonesia, Bangladesh and Vietnam is high to that of India. On the whole, Indian agriculture does not show high efficiency or productivity, though there is an improvement since independence.

The reasons and constrains of the above said results may have connections like**.,** population pressure, uneconomic holdings, uncertain monsoons and inadequate irrigation facilities, subsistence nature of farming, decline in soil fertility, lack of support services, poor organization of resources and lack of entrepreneurship and also poor crop plant population in

UNDER PEER REVIEW

case of broadcast sowing method resulting in uneven germination (upland and direct seeded lowland). Delay in monsoon onset often results in delayed and prolonged transplanting and sub-optimum plant population (mostly in rainfed low lands). Therefore, the productivity is low. In the high rainfed regions, the rain water is lost rapidly through deep percolation, because of the upland location and loose texture of the soil. In these soils the plant nutrient applied through fertilizers are lost rapidly and investment of fertilizer become risky. Further, low water retention capacity by the soil due to high permeability brings in moisture stress conditions quickly after cessation of rains. Such situation contributes low productivity.In the low rainfall regions, the crops suffer from iron and zinc deficiency in some soils. Among the above said problems and constrains, decrease in water availability leading to severe drought and the increasing areas under salinity are the major problems leading to severe yield reduction. Abiotic stress, which includes salinity, drought, heat and cold, critically threatens crop production and causes significant yield loss in large areas (Pareek*et al*, 2010; Mantri*et al*, 2012). Among these, soil salinity is the second major environmental constraint after drought, to crop production and is expected to increase due to global climate changes and as a consequence of many irrigation practices and depleting water resources respectively.Plant growth and developmental processes in terms of biochemical, physiological and morphological characteristics are inhibited by both water deficit and salt stresses (Hasegawa *et al*., 2000; Wang and Nil., 2001; Parida& Das, 2005).

Drought is an inevitable and recurring feature of world climate. Unlike any other disasters out there, predictions or early warning for drought can never be made. Despite our efforts to forecast its onset and modify its impact, drought remains the single most important factor affecting worldwide crop growth and productivity. More importantly, although plant drought response has been extensively studied, there are still no economically practical technological means to facilitate crop production under drought. Therefore, there is an urgent need to further understand and enhance crop tolerance to drought stress. Drought stress induces a number of changes at the morphological, physiological and biochemical level in all plant organs. Plants have evolved several strategies to cope with drought stress, including drought escape via a short life cycle or developmental plasticity, drought avoidance via enhanced water uptake and reduced water loss, as well as drought tolerance via osmotic adjustment, antioxidant capacity, and desiccation tolerance.

UNDER PEER REVIEW

Salinity is a common abiotic stress that severely limits crop growth and development, productivity and causes the continuous loss of arable land, which results in desertification in arid and semi-arid regions of the world (Pons *et al.*, 2011). It is estimated that more than 800 million hectares of land throughout the world are adversely affected by high salinity (Munns and Tester, 2008). Ali *et al*., (2013) opined that saline soils are characterized by excess of sodium ions with dominant anions of chloride and sulfate resulting in higher electrical conductivity (>4 dS m1). In general, salinity stress induces an initial osmotic stress and subsequent toxicity as a consequence of the accumulation of ions. However, damage can also ensue as a result of excessive reactive oxygen species (ROS) such as superoxide radicals (O_2), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH) produced at a high rate commonly accumulated in plant tissues due to ion imbalance and hyperosmotic stresses. ROS accumulation leads to lipid oxidation and has a negative effect on cellular metabolism and physiology, thus adversely ruining the membrane integrity (Munns*et al*., 2006). Salinity tolerance in glycophytic crops including rice is predominantly associated with the maintenance of ion homeostasis, particularly low Na^+/K^+ or high K^+/Na^+ ratios, through exclusion, compartmentation, and partitioning of Na^+ (Blumwald, 2000). In addition to ion homeostasis strategies, many plants have evolved mechanisms to regulate the synthesis and accumulation of compatible solutes such as proline and glycine betaine, which function as osmoprotectants and have a crucial role in plant adaptation to osmotic stress through stabilization of the tertiary structure of proteins (Munns and Tester, 2008). Rice is the most important global food crop that feeds over half of the world population and still getting a major proportion of their energy requirement from rice and its derived products with the demand for food expected to increase by another 38% within 30 years (Joseph *et al*., 2010). However, rice productivity in many areas is affected by salinity stress, which originates from the accumulation of underground salt and is exacerbated by salt mining, deforestation and irrigation (Akbar, 1986). Rice is generally characterized as a salt sensitive crop but the extent of its sensitivity varies during different growth and developmental stages. It is tolerant to salinity stress during germination and active tillering, whereas it displays more sensitivity during early vegetative and reproductive stages (Zhu *et al*., 2001).Attempts to measure the tolerance capacity in a genotypes or hybrid with single parameter have limited value because of the multiplicity of the factors and their interactions contributing to drought and salinity tolerance. Different researchers used different traits to appraise genetic variances in drought and salinity tolerance. Hence, in the present study, rice genotypes and hybrids have been selected to assess their tolerant

UNDER PEER REVIEW

capacity under drought and salinity and the various traits contributing to drought tolerance during germination and seedling establishment stage of the crop with the objectives viz., To investigate the germination, physiological and biochemical responses of rice genotypes to drought and salinity stress at germination level and To screen the rice genotypes that are tolerant and perform good to the induced drought and salinity stress conditions.

MATERIALS AND METHODS

The laboratory experiment was conducted in the Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore. Twenty six number of genotypes were selected for this experiments with three replications. Three treatments in water stress (T1: Control (water), T2: 1% mannitol and T3: 2% mannitol) and two treatments for salinity stress (T1: Control (water), T2: 200 mMNaCl) was imposed.

Varietal details

The experiment was conducted with twenty six rice genotypes *viz*.,IET 26487,IET 26493,IET 26498,IET 26866,IET 26871,IET 26908,IET 26910, IET 26913, IET 26917, GONTRABIDHAN-3, KARJAT-7, LALAT, MTU-1010, PR-113, US-312, BPT-2595, BPT-2593, BPT-2595, BPT-2766, BPT-2776 and BPT-2782 lines were used and CB06-803, CB13-804, CB13-805 and TNRH 55from TNAU.The seeds for the experiment were obtained from Department of Rice, Tamil Nadu Agricultural University, Coimbatore.

Methodology

The experiment was laid out under laboratory with 26 rice genotypes with three replications. The following stress treatments were imposed such as, water stress, NaCl stress and low temperature stress. The standardized protocols using Mannitol 1 % and 2% for creating the water stress and NaCl stress (200mM equivalent to -1.26 Mpa water potential). Change solutions once in every two days for water stress, NaCl stress. Drain completely rinses three to four times with fresh solutions if possible so as to avoid increased stress level due to Mannitol and NaCl. Experiment was continued at lab for one month and seedling vigour, germination and other related parameters were observed against control.

Observations Recorded

UNDER PEER REVIEW

The morphological, physiological and biochemical parameters were recorded at different date of stress imposition of rice seeds. Three plants in each replication were selected at random as sample seedlings and tagged to record observations in water and salinity stress. The methods followed in recording each of these parameters are described below.

Morphological and growth characters

Germination percentage

Seed germination tests were carried out and performed with three replications of 25 seeds each petriplates. The mean values were expressed in percentage.

Root length

The root length was measured and expressed as cm.

Shoot length

The shoot length was measured and expressed as cm.

Vigour Index

The Vigour Index was computed as per the procedure suggested by Abdul-Baki and Anderson, (1973) by using the following formula.

(Shoot length + Root length) x Germination percentage

UNDER PEER REVIEW

Stress Tolerance Index (STI)

The **Stress Tolerance Index** was computed as per the procedure suggested by Dhopte and Livera, (1989) by using the following formula.

Vigour Index (Treated)

STI = ---------- x 100

Vigour Index (Absolute control)

Physiological and biochemical parameters

Soluble protein content

Soluble protein content of the leaf was estimated by following the procedure described by Lowry *et al*. (1950) and expressed as mg g^{-1} of fresh weight.

Determination of soluble protein content

Proteins were estimated using Lowry *et al.* (1951) method.0.50 g of seed sample is macerated with 10ml of phosphate buffer (pH 7.0). The extract was centrifuged at 3000 rpm for 10 minutes and the supernatant was collected. One ml of the supernatant is transferred to a tube and 5ml of alkaline copper tartarate (ACT) reagent is added. The solution is kept as such for 30 minutes for color development. Then, 0.5ml of phenol reagent is added and the OD value of the sample for the intensity of blue color is read at 660nm in spectrophotometer. The protein content is expressed as mg g^{-1} of sample. Protein concentration was measured using bovine serum albumin as standard.

Alpha amylase content

Alpha amylasecontent of the germinated seedlings was estimated by following the procedure described by Miller, (1959) and expressed as enzyme unit g^{-1} fresh weight.

Determination of alpha amylase content

One g of sprouting cereal(here rice) is milled in 5ml of prechilled 0.05M citrate buffer, pH 6.0; the resulting homogenate is centrifuged at 10,000rpm for 10 minutes. Enzyme activity is assayed in the supernatant as yield of crude enzyme. 0.1ml of this supernatant is pipette into a separate test tube and 0.9ml of 2% soluble starch is added and is incubated in shaking water bath at $50^{O}C$ for 30 minutes. The reaction is stopped by adding DNSA reagent and boiled for at least 3 minutes for color development. Absorbance is read at 550nm against blank. Standard glucose curve is prepared from a series of glucose concentrations.

UNDER PEER REVIEW

Nitrate reductase activity

Nitrate reductase activity in young leaves was estimated as per the method described by Nicholas *et al.* (1976) and the enzyme activity was expressed as μg NO_2 g^{-1} h^{-1}.

Determination of nitrate reductase activity

A seedling of one g weight is taken in a test tube and 5ml of assay medium is added. This is kept inside the vacuum desiccator for 5 minutes, undisturbed. 2ml of the supernatant is pippeted out into a test tube and 1ml of 1M zinc acetate is added followed by 1ml of 70% ethanol. This is filtered using watman filter paper. To the filtrate 1ml of 1% sulphanilamide and 1ml of 0.02% NEDD is added. The OD Value is read at 540nm after the development of pink color using the spectrophotometer.

RESULTS AND DISCUSSION

Effect of drought and salinity on Morphological Characters

Germination Percentage (%)

The germination percentage was reduced by the drought and salinity compared to control. Significant difference was noticed in all the treatments with respect to seed germination. Control recorded the highest germination percentage (92 to 100%) and mannitol 1% (86 to 100%), mannitol 2% (70 to 100%) and salinity (74 to 98%) recorded least germination percentage **(Table 1)**. Among the rice genotypes, IET 26913 and BPT-2593 recorded the minimum germination percentage (92 %) followed by PR-113 (96%) under controlled conditions. The lowest rate of reduction in germination percentage in the genotype Karjat-7 and IET 26871 under mannitol stress imposed. Under salinity (NaCl 200 mM) conditions, the lowest rate of reduction in germination percentage recorded in the genotypes IET 26487, IET 26498, IET 26866, Karjat-7 and LALAT. Highest reduction was observed in BPT-2593 and IET-26913among the genotypes studied.

Seed germination is very important stage for the victorious establishment of vigorous seedlings.This germination stage is very sensitive todrought and salinity as compared to other stages. Salinity accumulates the toxic ion in plant cells causing a membrane damage and mineral imbalance. The most evident effect of drought and salinity to the seed germination of rice was reducing the osmotic potential of soil which makes decline in water imbibition by seed

UNDER PEER REVIEW

(Khan and Weber, 2008) to the creation of ionic toxicity which alters enzymes action involved in nucleic acid metabolism. Other impacts of salt stress on seed germination include change in metabolism of protein (Rasheed, 2009). In this present study, a significant increase was observed in germination percentage under controlled conditions. However, a considerable reduction could also be noticed in germination percentage due to the influence of mannitol and sodium chloride treatments. The genotypes Karjat-7 and IET 26871maintained its superiority with about 10 to 12 percent reduction, whereas, all the other genotypes showed about 25 to 30 percent reduction due to drought and salinity.

Shoot and root length (cm)

The results of shoot and root length showed a significant reduction under drought and salinity stress compared to controlled condition **(Table 1).**

Among the rice genotypes, IET 26498 recorded the maximum shoot length (11.9cm) followed by Lalat (11.8cm) and MTU-1010 (11.7cm) under controlled conditions. The lowest rate of reduction in shoot length due to the influence of mannitol and sododium chloride in the genotypes of IET-26866, CB13-804 under water stress and the genotypes CB-13-805, IET-26871 had lowest reduction in shoot length under salinity stress imposed and the highest reduction was observed in GONTRABADAN and US-312among the rice genotypes and hybrids studied.

The root length was reduced by the influence of mannitol and sodium chloride compared to control. Significant differences were noticed in all the treatments and genotypes with respect to root length. The control was recorded the maximum mean root length (11.5 cm) and the mannitol 1%, mannitol 2% and sodium chloride 200 mM recorded the minimum mean root length (7.4cm, 8.9cm and 4.0 cm) **(Table 2).**

Among the rice genotypes, IET-26498, CB-06-803, CB13-805, Lalat and IET- 26871 recorded the maximum root length (9.8 to 10.0 cm) under water stressed (mannitol 1 to 2%)conditions and genotypes IET-26493 and CB-13-804 had maximum root length under salinity stress imposed. The rate of reduction in root length was the lowest in the genotypes IET-26498 and CB-06-803 under water stress (Mannitol) imposed and the highest reduction was observed in GONTRABADAN and IET-26917 among the genotypes and hybrids studied.

UNDER PEER REVIEW

The main functions of root are absorption of water and inorganic nutrients and anchoring of the plant body to the ground.Many researchers reported that with an increase in drought and salinity there was a decrease in the development of the xylem and phloem. Many studies have shown that the fresh and dry weights of the shoot system are affected, either negatively or positively, by changes in salinity concentration, type of salt present, or type of plant species (Taffouo*et al*., 2010). Root length was more suppressed than shoot by salinity at each specific salt concentration level. According to Rahman*et al*., (2001), who stated that, the gradual decrease in root length with the increase in salinity as observed might be due to more inhibitory effect of combination of drought and NaCl salt to root growth compared to that of shoot growth. In this present study, a lesser reduction was observed in the genotypes IET-26498 and CB-06-803 under water stress (Mannitol) imposed showed about 11 to 15 percent and the highest reduction was observed in GONTRABADAN and IET-26917 with about 22 to 31 percent reduction due to the impact of drought and salinity.

Vigour index

Significant differences were noticed in all treatments and genotypes with respect to vigour index. The highest mean vigour index was recorded in control (2184.4) and lowest mean vigour index was recorded by salinity (802.1) **(Table 2).**

Among the rice genotypes, CB-06-803, Lalat and IET- 26498 recorded the maximum vigour index (2069.2.0 to 2222.5) (1731.2 to 2040.5) under the influence of drought stressed (mannitol 1 to 2%) conditions and genotypes IET-26866 and IET-26871 had maximum vigour index under salinity stress (NaCl 200mM) imposed. The rate of reduction in vigour index was the lowest in the genotypes CB-06-803, Lalat and IET- 26498 under water stress (Mannitol) imposed and the highest reduction was observed in TNRH-55among the genotypes and hybrids studied.

Stress Tolerance Index

The highest mean Stress Tolerance Index was recorded in control (83) and lowest mean Stress Tolerance Index was recorded by salinity (36.8) (Table 3).

Among the rice genotypes, Lalat recorded the maximum Stress Tolerance Index (87.8 and 71.8) under the influence of drought stressed of mannitol 1 and 2% conditions which was followed by CB-06-803 (87.4 and 70.8) and IET-26866 (87.4 and 84.0) and IET-26871 had

UNDER PEER R

maximum Stress Tolerance Index under salinity stress (NaCl 200mM) imposed among the genotypes and hybrids studied.

Effect of water and salinity stress on Physiological and biochemical parameters

Soluble Protein Content (mg/g)

The data on soluble protein content was reduced by the influence of mannitol and sodium chloride compared to control. The control was recorded the maximum mean soluble protein content (19.7 mg g^{-1}) and the mannitol 1%, mannitol 2% and sodium chloride 200 mM recorded the minimum mean soluble protein content (16.4mg g^{-1}, 14.2mg g^{-1} and 7.2mg g^{-1}) **(Table 3).**

Among the rice genotypes, Lalat recorded the maximum soluble protein content (20.0 to 16.4 mg g^{-1}) under water stressed (mannitol 1 to 2%) conditions which was followed by IET 26498 (18.6mg g^{-1} and 18.4mg g^{-1}) and CB-06-803 (19.2mg g^{-1} and 15.6mg g^{-1}) and genotypes IET-26866 and IET-26871 had maximum soluble protein content (11.7 mg g^{-1}) under salinity stress imposed. The rate of reduction in soluble protein content was the lowest in the genotypes IET-26866 and CB-06-803 under water stress (Mannitol) imposed and the highest reduction was observed in BPT-2776 and BPT-2411among the genotypes and hybrids studied.

The soluble protein content of the rice seedlings, being a measure of RuBP carboxylase activity was considered as an index for photosynthetic efficiency. There were reports that RuBP-case enzyme forms nearly 80% of the soluble proteins in leaves of many plants (Joseph *et al.*, 1981). Diethelm and Shibles (1989) opined that the RUBISCO content per unit leaf area was positively correlated with that of soluble protein content of the leaf. Soluble protein content was estimated in order to find out the photosynthetic capacity of rice genotypes under water deficit situations. In the present study, drought and salinity caused a significant reduction in soluble protein content of seedlings of all the rice genotypes. Among the genotypes studied, Lalat, IET 26498and CB-06-803 maintained its superiority with about 8 to 12 percent reduction in soluble protein content due to the influence of mannitol and sodium chloride. The mechanism of reduction in soluble protein content due to water deficit by reduction in RUBISCO enzyme activity leads to lower CO_2 assimilation. These findings were in accordance with the results of Chaves *et al.* (2009) who observed a significant reduction in soluble protein content of rice plants grown under drought and salinity stress conditions. Besides these results, Martignone*et al*. (1987) observed that in soybean soluble protein content was the first nitrogenous compound affected under abiotic stress conditions, which at severity got denatured and lost the activity.

UNDER PEER REVIEW

Hsiao (1974) reported that the decrease in the protein level in water stressed plants could be attributed to decrease in protein synthesis, the decreased availability of amino acids and denaturation of the enzymes involved in amino acid and protein synthesis, perhaps at the ribosomal level.

Nitrate reductase enzyme activity(µg g^{-1} d^{-1})

The result on nitrate reductase enzyme activity was reduced by the influence of mannitol and sodium chloride compared to control. The control was recorded the maximum mean nitrate reductase enzyme activity (152.9µg g^{-1} d^{-1}) and the mannitol 1%, mannitol 2% and sodium chloride 200 mM recorded the minimum mean nitrate reductase enzyme activity (127.2µg g^{-1} d^{-1}, 110.1µg g^{-1} $d^{-1}$and 56.1µg g^{-1} d^{-1}) **(Table 4).**

Among the rice genotypes, Lalat recorded the maximum nitrate reductase enzyme activity (149.6µg g^{-1} d^{-1}) under water stressed (mannitol 1%) conditions which was followed by IET 26498 (144.8µg g^{-1} d^{-1}) and IET 26498 (144.4µg g^{-1} d^{-1}) and genotypes IET-26866 had maximum nitrate reductase enzyme activity (91.1µg g^{-1} d^{-1}) under salinity stress imposed. The rate of reduction in nitrate reductase enzyme activity was the lowest in the genotypes CB-06-803 under water stress (Mannitol) imposed and the highest reduction was observed in BPT-2593 and BPT-2411 among the genotypes and hybrids studied.

Nitrate reductase is the important key enzyme of the nitrogen assimilation pathway in plant system. Its catalytic action in tissue is subjected to complex regulation in response to different abiotic factors. Drought and salinity is one of the most important abiotic factors that restrictions growth and development of plants. It is well known that initial steps of nitrogen metabolism in plants (NO_3^- ions uptake, reduction of nitrates and assimilation of ammonium) are sensitive to drought and salt stress (Flores, 2004). The plants are treated with NaCl reduces the level of nitrate ions in the cytoplasm since it also inhibits nitrate uptake by plants (Klobus*et al.*,1988). In the present study, drought and salinity caused a significant reduction in nitrate reductase enzyme activity of seedlings of all the rice genotypes. Among the genotypes studied, Lalat, IET 26498and CB-06-803 maintained its superiority with about 10 to 14 percent reduction in nitrate reductase enzyme activity due to the influence of mannitol and sodium chloride. These findings were in accordance with the results ofRao and Gnaham, (1990), who stated that, the nitrate uptake and nitrate reductase activity (NRA) decrease in plants under drought and salinity stress.

UNDER PEER REVIEW

Alpha amylase enzyme activity (enzyme unit g^{-1} fresh weight)

The data on alpha amylase enzyme activity was reduced by the influence of mannitol and sodium chloride compared to control. The control was recorded the maximum mean alpha amylase enzyme activity (13.8) and the mannitol 1%, mannitol 2% and sodium chloride 200 mM recorded the minimum mean alpha amylase enzyme activity (11.4, 9.9and 5.1) **(Table 4).**

Among the rice genotypes, Lalat recorded the maximum alpha amylase enzyme activity (14.0) under water stressed (mannitol 1 to 2%) conditions which was followed by CB-06-803 (13.4) and genotypes IET-26493 and IET-26487 had maximum alpha amylase enzyme activity (6.7 and 6.2) under salinity stress imposed.

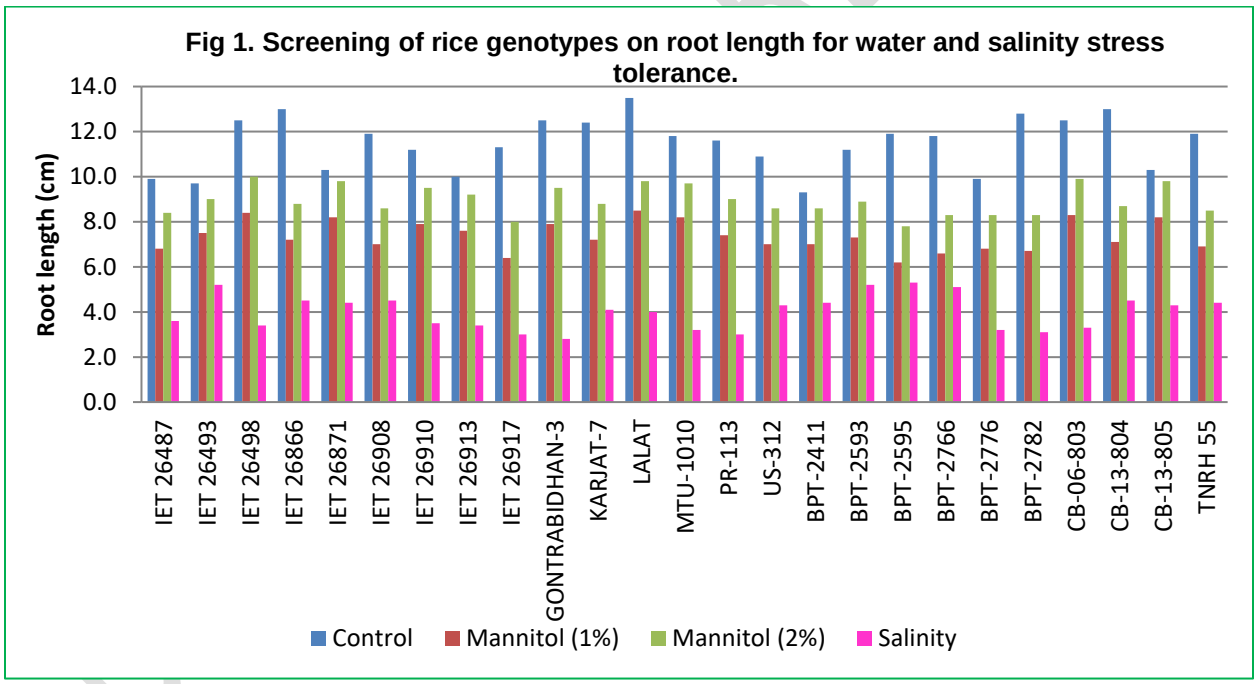
Higher accumulation of alpha-amylase during grain development, especially the filling stage, could result in rice grains with reduced quality, chalky, and with few stored starch grains. In rice, seed germination is dependent on the degradation of storage reserves in mature seeds, and the sugars from starch hydrolysis are the major source of energy for seedling emergence (Beck and Ziegler, 1989). α-Amylase is the important enzyme implicated in starch mobilization; thus, α-amylase enzyme activity is an important factor in seed germination (Karrer *et al*., 1993). In this study, drought and salinity caused a significant reduction in alpha-amylase enzyme activity of seedlings of all the rice genotypes. Among the genotypes studied, Lalat, IET 26498and CB-06-803 maintained its superiority with about 12 to 17 percent reduction in alpha-amylase enzyme activity due to the influence of mannitol and sodium chloride. These findings were in accordance with the results of Appleford *et al*. (2008), who found that, positive relationships between bioactive GA content and α-amylase activity and between α-amylase activity and the rice seed germination rate and the seeds are growing under drought and NaCl induced bioactive GA deficiency inhibits seed germination by decreasing α-amylase activity.

Conclusion:

The lowest rate of reduction in germination percentage recorded in the genotypes IET 26487, IET 26498, IET 26866, Karjat-7 and LALAT. The lowest rate of reduction in shoot length due to the influence of mannitol and sododium chloride in the genotypes of IET-26866, CB13-804 under water stress and the genotypes CB-13-805, IET-26871 had lowest reduction in shoot length under salinity stress CB-06-803, Lalat and IET- 26498 recorded the maximum vigour index (2069.2.0 to 2222.5) (1731.2 to 2040.5) under the influence of drought stressed (mannitol 1 to 2%) conditions and genotypes IET-26866 and IET-26871 had maximum vigour index under

UNDER PEER REVIEW

salinity stress (NaCl 200mM). Lalat recorded the maximum Stress Tolerance Index (87.8 and 71.8) under the influence of drought stressed of mannitol 1 and 2% conditions which was followed by CB-06-803 (87.4 and 70.8) and IET-26866 (87.4 and 84.0) and IET-26871 had maximum Stress Tolerance Index under salinity stress (NaCl 200mM). Lalat recorded the maximum soluble protein content (20.0 to 16.4 mg g^{-1}) under water stressed (mannitol 1 to 2%) conditions which was followed by IET 26498 (18.6mg g^{-1} and 18.4mg g^{-1}) and CB-06-803 (19.2mg g^{-1} and 15.6mg g^{-1}) and genotypes IET-26866 and IET-26871 had maximum soluble protein content (11.7 mg g^{-1}) under salinity stress imposed. Lalat recorded the maximum nitrate reductase enzyme activity (149.6µg g^{-1} d^{-1}) under water stressed (mannitol 1%) conditions which was followed by IET 26498 (144.8µg g^{-1} d^{-1}) and IET 26498 (144.4µg g^{-1} d^{-1}) and genotypes IET-26866 had maximum nitrate reductase enzyme activity (91.1µg g^{-1} d^{-1}) under salinity stress imposed.



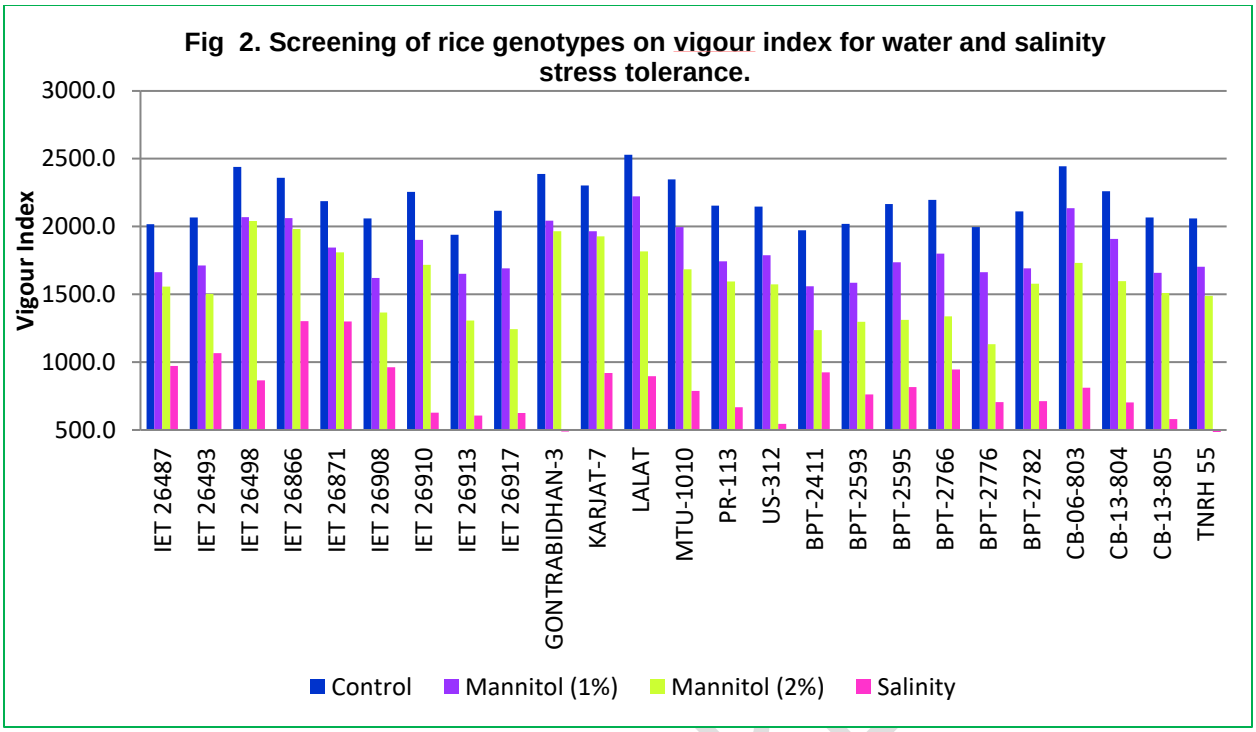


Fig 2. Screening of rice genotypes on vigour index for water and salinity stress tolerance.
Vigour Index
3000.0
2500.0
2000.0
1500.0
1000.0
500.0
IET 26487
IET 26493
IET 26498
IET 26866
IET 26871
IET 26908
IET 26910
IET 26913
IET 26917
GONTRABIDHAN-3
KARJAT-7
LALAT
MTU-1010
PR-113
US-312
BPT-2411
BPT-2593
BPT-2595
BPT-2766
BPT-2776
BPT-2782
CB-06-803
CB-13-804
CB-13-805
TNRH 55
Control
Mannitol (1%)
Mannitol (2%)
Salinity

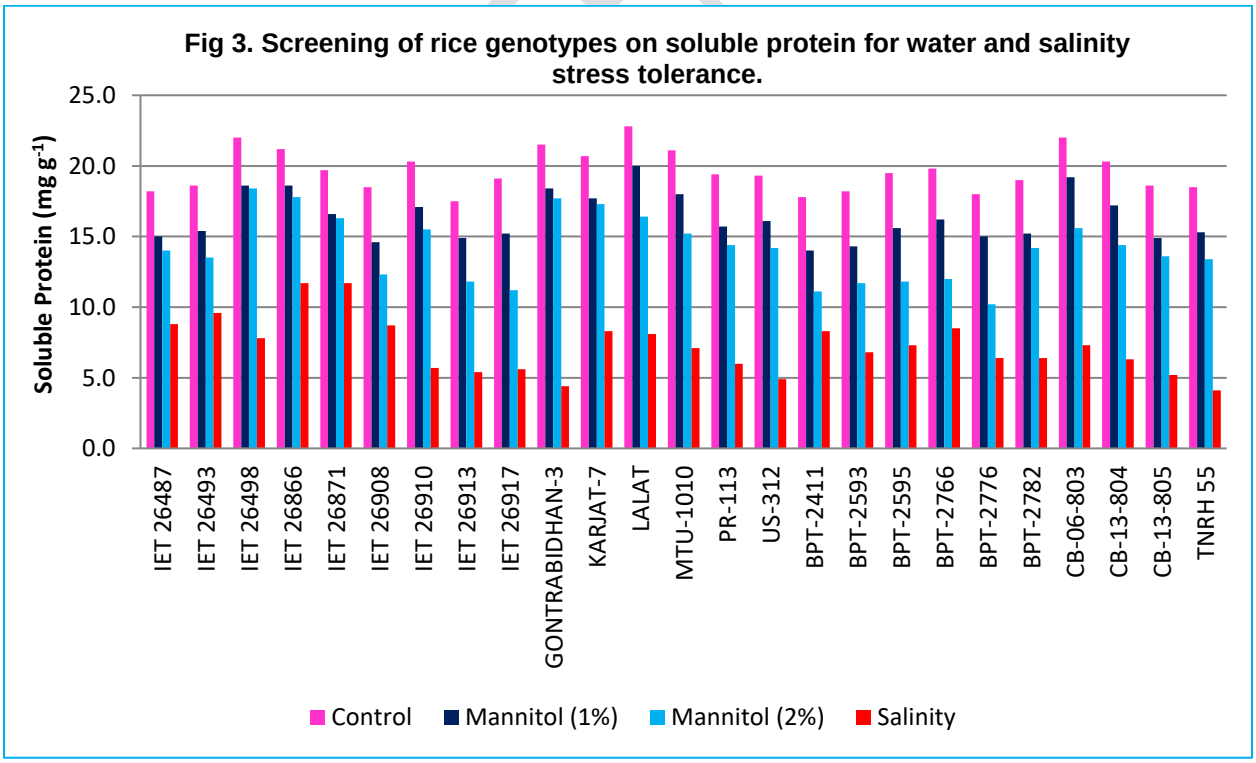


Fig 3. Screening of rice genotypes on soluble protein for water and salinity stress tolerance.
Soluble Protein (mg g-1)
25.0
20.0
15.0
10.0
5.0
0.0
IET 26487
IET 26493
IET 26498
IET 26866
IET 26871
IET 26908
IET 26910
IET 26913
IET 26917
GONTRABIDHAN-3
KARJAT-7
LALAT
MTU-1010
PR-113
US-312
BPT-2411
BPT-2593
BPT-2595
BPT-2766
BPT-2776
BPT-2782
CB-06-803
CB-13-804
CB-13-805
TNRH 55
Control
Mannitol (1%)
Mannitol (2%)
Salinity

Fig 4. Screening of rice genotypes on alpha amylase for water and salinity stress tolerance.

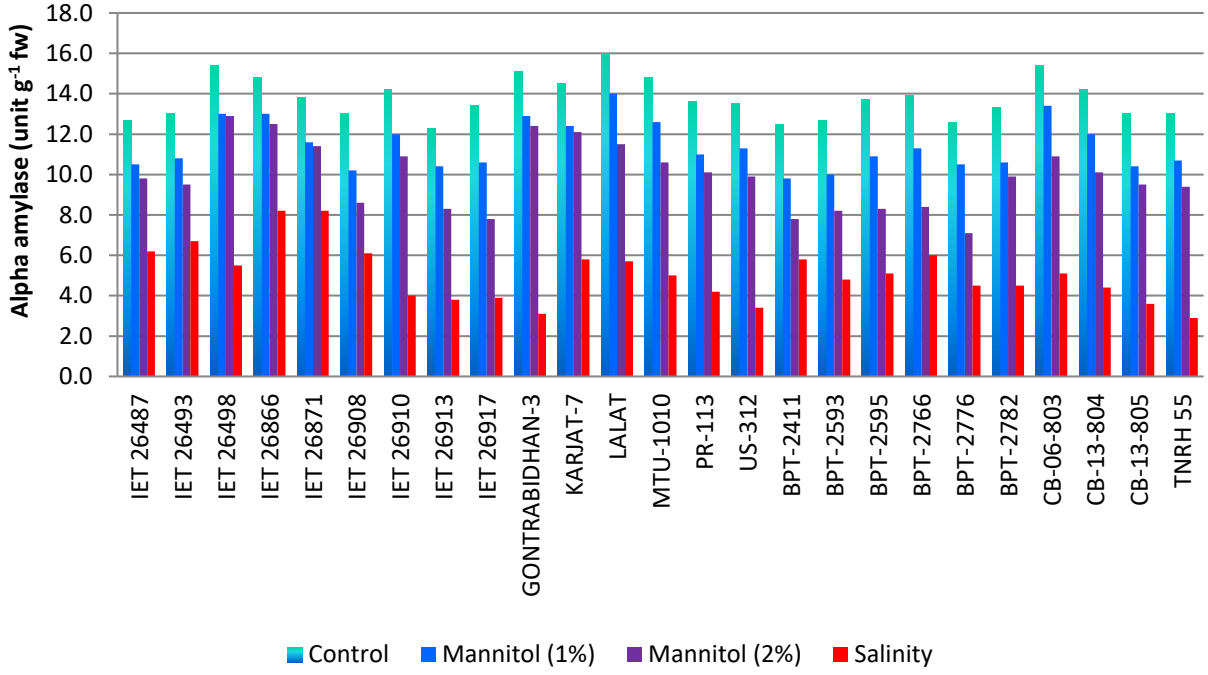
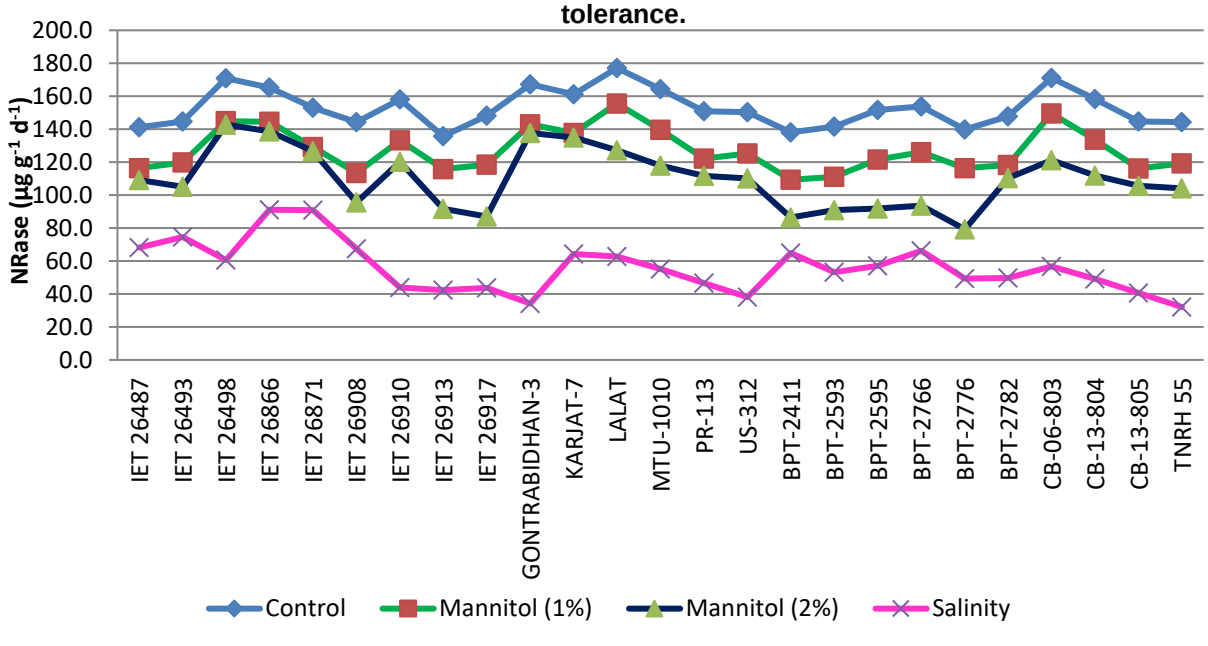


Fig 5. Screening of rice genotypes on NRase for water and salinity stress tolerance.



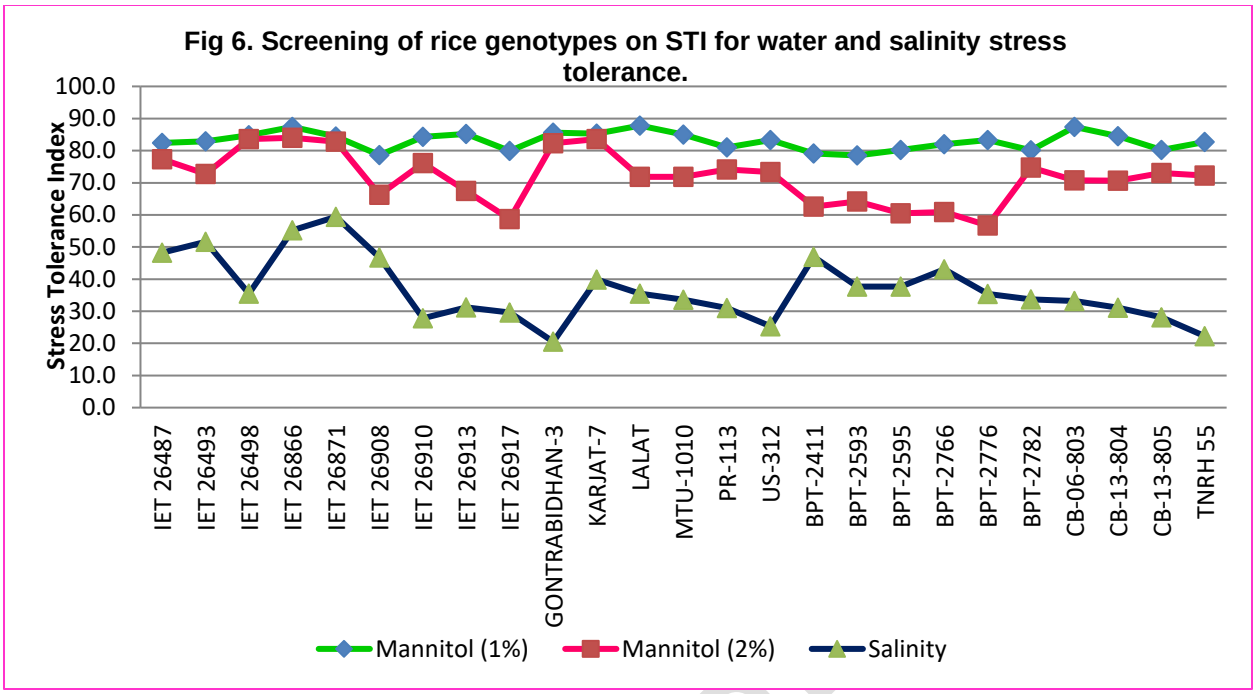


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Table 1.Screening of rice genotypes on seed germination and shoot length for water and salinity stress tolerance.

Variety / Treatments	Seed Germination (%)				Shoot length (cm)			
	Control	Mannitol (1%)	Mannitol (2%)	Salinity	Control	Mannitol (1%)	Mannitol (2%)	Salinity
IET 26487	100.0	100.0	96.0	98.0	10.3	9.9	7.9	6.3
IET 26493	100.0	100.0	90.0	96.0	11.0	9.7	7.7	5.9
IET 26498	100.0	98.0	98.0	98.0	11.9	12.8	10.9	5.5
IET 26866	100.0	100.0	98.0	98.0	10.6	13.5	11.5	8.8
IET 26871	100.0	100.0	100.0	96.0	11.6	10.3	8.3	9.2
IET 26908	92.0	86.0	74.0	78.0	10.5	11.9	9.9	7.9
IET 26910	100.0	100.0	92.0	88.0	11.4	11.2	9.2	3.7
IET 26913	92.0	94.0	76.0	74.0	11.1	10.0	8.0	4.8
IET 26917	100.0	96.0	72.0	94.0	9.9	11.3	9.3	3.7
GONTRABIDHAN-3	100.0	100.0	98.0	86.0	11.4	12.6	10.6	2.9
KARJAT-7	100.0	100.0	100.0	98.0	10.6	12.5	10.5	5.3
LALAT	100.0	100.0	84.0	98.0	11.8	13.8	11.8	5.2
MTU-1010	100.0	100.0	86.0	92.0	11.7	11.8	9.9	5.4
PR-113	96.0	92.0	86.0	86.0	10.8	11.6	9.6	4.8
US-312	100.0	100.0	90.0	80.0	10.6	10.9	8.9	2.5
BPT-2411	100.0	96.0	78.0	82.0	10.4	9.3	7.3	6.9
BPT-2593	92.0	86.0	72.0	74.0	10.8	11.2	9.2	5.1
BPT-2595	100.0	96.0	74.0	80.0	9.8	11.9	9.9	4.9
BPT-2766	100.0	98.0	74.0	84.0	10.2	11.8	9.8	6.2
BPT-2776	100.0	100.0	70.0	86.0	10.1	9.9	7.9	5.0
BPT-2782	92.0	86.0	82.0	86.0	10.1	13.0	11.0	5.2
CB-06-803	100.0	98.0	94.0	92.0	11.8	12.8	10.9	5.4
CB-13-804	100.0	98.0	92.0	90.0	10.6	13.5	11.5	8.7
CB-13-805	100.0	98.0	94.0	90.0	11.5	10.3	8.3	9.1
TNRH 55	100.0	98.0	92.0	90.0	10.5	11.9	9.9	7.8
Mean	**98.6**	**96.8**	**86.5**	**88.6**	**10.8**	**11.5**	**9.6**	**5.8**
SEd	**1.91**	**5.46**	**1.73**	**1.71**	**0.20**	**0.23**	**0.19**	**0.12**
CD (0.05)	**3.95**	**11.24**	**3.57**	**3.53**	**0.43**	**0.47**	**0.39**	**0.24**

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Table 2.Screening of rice genotypes on root length and seedling vigour for water and salinity stress tolerance.

Variety / Treatments	Root length (cm)				Seedling vigour			
	Control	Mannitol (1%)	Mannitol (2%)	Salinity	Control	Mannitol (1%)	Mannitol (2%)	Salinity
IET 26487	9.9	6.8	8.4	3.6	2016.7	1662.5	1558.4	972.3
IET 26493	9.7	7.5	9.0	5.2	2066.7	1712.5	1501.9	1065.6
IET 26498	12.5	8.4	10.0	3.4	2440.0	2069.2	2040.5	866.9
IET 26866	13.0	7.2	8.8	4.5	2360.0	2062.5	1981.7	1302.1
IET 26871	10.3	8.2	9.8	4.4	2186.7	1845.8	1810.0	1299.2
IET 26908	11.9	7.0	8.6	4.5	2060.8	1620.2	1365.3	963.2
IET 26910	11.2	7.9	9.5	3.5	2256.7	1902.5	1717.3	628.3
IET 26913	10.0	7.6	9.2	3.4	1939.1	1652.6	1308.0	605.5
IET 26917	11.3	6.4	8.0	3.0	2116.7	1692.0	1243.2	625.5
GONTRABIDHAN-3	12.5	7.9	9.5	2.8	2386.7	2042.5	1965.3	488.9
KARJAT-7	12.4	7.2	8.8	4.1	2303.3	1965.8	1926.7	919.1
LALAT	13.5	8.5	9.8	4.0	2530.0	2222.5	1817.2	897.1
MTU-1010	11.8	8.2	9.7	3.2	2346.7	1995.8	1684.0	788.1
PR-113	11.6	7.4	9.0	3.0	2153.6	1744.2	1595.1	666.7
US-312	10.9	7.0	8.6	4.3	2146.7	1789.2	1573.7	544.0
BPT-2411	9.3	7.0	8.6	4.4	1973.3	1560.8	1236.2	925.1
BPT-2593	11.2	7.3	8.9	5.2	2020.9	1585.8	1298.4	760.9
BPT-2595	11.9	6.2	7.8	5.3	2166.7	1736.8	1311.1	816.0
BPT-2766	11.8	6.6	8.3	5.1	2196.7	1801.2	1338.1	946.4
BPT-2776	9.9	6.8	8.3	3.2	1996.7	1662.5	1132.8	706.4
BPT-2782	12.8	6.7	8.3	3.1	2111.7	1690.7	1577.5	712.1
CB-06-803	12.5	8.3	9.9	3.3	2444.0	2136.5	1731.2	811.1
CB-13-804	13.0	7.1	8.7	4.5	2260.7	1909.8	1598.0	702.1
CB-13-805	10.3	8.2	9.8	4.3	2067.6	1658.2	1509.1	580.7
TNRH 55	11.9	6.9	8.5	4.4	2060.7	1703.2	1487.7	458.0
Mean	**11.5**	**7.4**	**8.9**	**4.0**	**2184.4**	**1817.0**	**1572.3**	**802.1**
SEd	**0.23**	**0.14**	**0.17**	**0.07**	**42.45**	**35.20**	**31.88**	**15.88**
CD (0.05)	**0.47**	**0.29**	**0.35**	**0.15**	**87.43**	**72.50**	**65.66**	**32.72**

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Table 3.Screening of rice genotypes on stress tolerance index and soluble protein content for water and salinity stress tolerance.

Variety / Treatments	Stress Tolerance Index (%)			Soluble protein (mg g^{-1})			
	Mannitol (1%)	Mannitol (2%)	Salinity	Control	Mannitol (1%)	Mannitol (2%)	Salinity
IET 26487	82.4	77.3	48.2	18.2	15.0	14.0	8.8
IET 26493	82.9	72.7	51.6	18.6	15.4	13.5	9.6
IET 26498	84.8	83.6	35.5	22.0	18.6	18.4	7.8
IET 26866	87.4	84.0	55.2	21.2	18.6	17.8	11.7
IET 26871	84.4	82.8	59.4	19.7	16.6	16.3	11.7
IET 26908	78.6	66.3	46.7	18.5	14.6	12.3	8.7
IET 26910	84.3	76.1	27.8	20.3	17.1	15.5	5.7
IET 26913	85.2	67.5	31.2	17.5	14.9	11.8	5.4
IET 26917	79.9	58.7	29.6	19.1	15.2	11.2	5.6
GONTRABIDHAN-3	85.6	82.3	20.5	21.5	18.4	17.7	4.4
KARJAT-7	85.3	83.6	39.9	20.7	17.7	17.3	8.3
LALAT	87.8	71.8	35.5	22.8	20.0	16.4	8.1
MTU-1010	85.0	71.8	33.6	21.1	18.0	15.2	7.1
PR-113	81.0	74.1	31.0	19.4	15.7	14.4	6.0
US-312	83.3	73.3	25.3	19.3	16.1	14.2	4.9
BPT-2411	79.1	62.6	46.9	17.8	14.0	11.1	8.3
BPT-2593	78.5	64.2	37.7	18.2	14.3	11.7	6.8
BPT-2595	80.2	60.5	37.7	19.5	15.6	11.8	7.3
BPT-2766	82.0	60.9	43.1	19.8	16.2	12.0	8.5
BPT-2776	83.3	56.7	35.4	18.0	15.0	10.2	6.4
BPT-2782	80.1	74.7	33.7	19.0	15.2	14.2	6.4
CB-06-803	87.4	70.8	33.2	22.0	19.2	15.6	7.3
CB-13-804	84.5	70.7	31.1	20.3	17.2	14.4	6.3
CB-13-805	80.2	73.0	28.1	18.6	14.9	13.6	5.2
TNRH 55	82.7	72.2	22.2	18.5	15.3	13.4	4.1
Mean	**83.0**	**71.7**	**36.8**	**19.7**	**16.4**	**14.2**	**7.2**
SEd	**1.62**	**1.46**	**0.73**	**0.38**	**0.32**	**1.86**	**0.14**
CD (0.05)	**3.34**	**3.00**	**1.51**	**0.79**	**0.65**	**3.82**	**0.29**

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Table 4.Screening of rice genotypes on NRase and alpha amylase enzyme activity for water and salinity stress tolerance.

Variety / Treatments	Nitrate reductase activity (µg g^{-1} d^{-1})				Alpha amylase (enzyme unit g^{-1} fresh weight)			
	Control	Mannitol (1%)	Mannitol (2%)	Salinity	Control	Mannitol (1%)	Mannitol (2%)	Salinity
IET 26487	141.2	116.4	109.1	68.1	12.7	10.5	9.8	6.2
IET 26493	144.7	119.9	105.1	74.6	13.0	10.8	9.5	6.7
IET 26498	170.8	144.8	142.8	60.7	15.4	13.0	12.9	5.5
IET 26866	165.2	144.4	138.7	91.1	14.8	13.0	12.5	8.2
IET 26871	153.1	129.2	126.7	90.9	13.8	11.6	11.4	8.2
IET 26908	144.3	113.4	95.6	67.4	13.0	10.2	8.6	6.1
IET 26910	158.0	133.2	120.2	44.0	14.2	12.0	10.9	4.0
IET 26913	135.7	115.7	91.6	42.4	12.3	10.4	8.3	3.8
IET 26917	148.2	118.4	87.0	43.8	13.4	10.6	7.8	3.9
GONTRABIDHAN-3	167.1	143.0	137.6	34.2	15.1	12.9	12.4	3.1
KARJAT-7	161.2	137.6	134.9	64.3	14.5	12.4	12.1	5.8
LALAT	177.1	155.6	127.2	62.8	16.0	14.0	11.5	5.7
MTU-1010	164.3	139.7	117.9	55.2	14.8	12.6	10.6	5.0
PR-113	150.8	122.1	111.7	46.7	13.6	11.0	10.1	4.2
US-312	150.3	125.2	110.2	38.1	13.5	11.3	9.9	3.4
BPT-2411	138.1	109.3	86.5	64.8	12.5	9.8	7.8	5.8
BPT-2593	141.5	111.0	90.9	53.3	12.7	10.0	8.2	4.8
BPT-2595	151.7	121.6	91.8	57.1	13.7	10.9	8.3	5.1
BPT-2766	153.8	126.1	93.7	66.2	13.9	11.3	8.4	6.0
BPT-2776	139.8	116.4	79.3	49.4	12.6	10.5	7.1	4.5
BPT-2782	147.8	118.3	110.4	49.8	13.3	10.6	9.9	4.5
CB-06-803	171.1	149.6	121.2	56.8	15.4	13.4	10.9	5.1
CB-13-804	158.2	133.7	111.9	49.1	14.2	12.0	10.1	4.4
CB-13-805	144.7	116.1	105.6	40.6	13.0	10.4	9.5	3.6
TNRH 55	144.2	119.2	104.1	32.1	13.0	10.7	9.4	2.9
Mean	**152.9**	**127.2**	**110.1**	**56.1**	**13.8**	**11.4**	**9.9**	**5.1**
SEd	**2.97**	**2.46**	**2.23**	**1.11**	**0.27**	**0.22**	**0.20**	**0.10**
CD (0.05)	**6.12**	**5.07**	**4.59**	**2.29**	**0.55**	**0.46**	**0.42**	**0.21**

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Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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